Mortality in Juvenile Chinook Salmon Exposed to Sodium Pentachlorophenate and Undergoing Progressively Symptomatic Bacterial Kidney Disease

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MORTALITY IN JUVENILE CHINOOK SALMON EXPOSED TO SODIUM PENTACHLOROPHENATE AND UNDERGOING PROGRESSIVELY SYMPTOMATIC BACTERIAL KIDNEY DISEASE

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ABSTRACT

Iwama, G. K., and G. L. Greer. 1982. Mortality in juvenile chinook salmon exposed to sodium pentachlorophenate and undergoing progressively symptomatic bacterial kidney disease. Can. Tech. Rep. Fish. Aquat. Sci. No. 1100: iv + 9 p.

Juvenile chinook salmon were exposed continuously to sodium pentachlorophenate (NaPCP) at two levels (0.05 and 0.50 the 96 hr LC50 of 0.078 mg/L) after they were infected experimentally via intraperitoneal injection with kidney disease bacteria grown in culture. Five groups of fish received the toxicant and disease treatments, either singly or in combination; a sixth group of fish was maintained as a control. In the combined treatments, toxicant exposure at the 0.05 and 0.50 levels lagged the bacterial infection by 5 and 6 days, respectively. Progression of bacterial kidney disease was quantitated by assignment of a symptomatic index based on the number and degree of external and internal disease symptoms present. Few mortalities occurred in fish exposed for five weeks to the 0.05 and 0.50 levels of the toxicant alone. Mortalities were higher in the disease and disease plus 0.05 level toxicant treatments with increased mortality starting at a symptomatic index of about 3 (max. = 6 for terminal stage of disease) and occurring 24-25 days post-injection. A marked synergism was apparent when treatment with NaPCP at 0.50 the 96 hr LC50 was combined with bacterial kidney disease. All fish in this treatment group died on the 14th day post-injection and the 8th day of toxicant exposure. The symptomatic index of these fish was approximately 2. Physical signs at this stage of bacterial kidney disease were not pronounced, consisting of a moderately distended abdomen and slight enlargement of the kidney and hind gut.

Key words: toxicity, bacterial kidney disease, juvenile chinook salmon, pentachlorophenate.

RÉSUMÉ

Iwama, G. K., and G. L. Greer. 1982. Mortality in juvenile chinook salmon exposed to sodium pentachlorophenate and undergoing progressively symptomatic bacterial kidney disease. Can. Tech. Rep. Fish. Aquat. Sci. 1100: iv + 9 p.

On a exposé continuellement de jeunes saumons quinnats à deux concentrations de pentachlorophénate de sodium (NaPCP), c'est-à-dire à 0,05 et 0,50 fois la CL50 96 h de 0,078 mg/L, après leur avoir incolé expérimentalement, au moyen d'une injection intrapéritionéale, des bactéries de la maladie du rein provenant de cultures. Cinq groups de poissons ont été exposés à la substance toxique ou à la bactérie ou aux deux à la fois; un sixième groupe a servi de groupe-témoin. Chez les groupes soumis aux deux traitements, l'exposition à la substance toxique à des concentrations de 0,05 et de 0,50 a retardé de 5 et 6 jours respectivement la contagion bactérienne. On a quantifié la progression de la maladie batérienne du rein en attribuant un indice symptomatique fondé sur le nombre et la gravité des symptômes externes et internes. Peu de poissons sont morts d'avoir été exposés pendant cinq semaines aux concentrations de 0,05 et 0,50 de la substance toxique seulement. Les groupes exposés à la maladie et à la maladie plus à une concentration de substance toxique de 0,5 ont connu un plus grand nombre de mortalités, la mortalité commençant à augmenter lorsque l'indice symptomatique était de 3 (max. = 6 au stade terminal de la maladie) et entre 24 et 25 jours après l'injection. On a noté au synergisme prononcé lorsque, en plus d'exposer les poissons à une concentration de 0,50 fois la CL50 96 h de NaPCP, on leur a inoculé la maladie bactérienne de rein. Tous les poissons de ce groupe sont morts 14 jours après l'injection et le 8e jour après avoir été exposés à la substance toxique. L'indice symptomatique de ces poissons était d'environ 2. À ce stade de la maladie bactérienne du rein, les symptômes physiques étaient peu prononcés, consistant en un abdomen modérément dilaté et en une légère hypertrophie dr rein et du gros intestin.

Mots-clés: toxicité, maladie bactérienne du rein, jeune saumon quinnat, pentachlorophénate.

INTRODUCTION

In a previous communication (Iwama and Greer 1980) we reported the enhanced toxicity of sodium pentachlorophenate (NaPCP) to juvenile coho salmon (Oncorhynchus kisutch) with asymptomatic bacterial kidney disease (BKD). In that experiment the juvenile coho were infected experimentally via transmission in water. This was accomplished by holding healthy coho for 28 days in the downstream half of a tank which also contained in the upstream half an adult sockeye salmon taken from a known infected stock and exhibiting overt physical signs of BKD. When used subsequently in bioassay, the asymptomatic coho were approximately twice as sensitive to the toxic action of NaPCP (96 h LC50 = 0.039 ± 0.004 mg/L) compared to the controls (0.065 ± 0.003 mg/L). In another experiment (Iwama 1980), the incubation times producing 100% mortality were determined for juvenile coho infected via intraperitoneal injection with 0.1 mL suspensions of kidney disease bacteria grown in culture. Injected suspensions having optical densities of 0.25, 0.10, and 0.01 (420 nm) produced 100% mortality in 45, 49, and 58 days, respectively. The physical characteristics most commonly present in the fish at death (e.g. exopthalmia, distended abdomen, excessive intraperitoneal fluid, kidney lesions, gram positive diplobacilli in kidney smears) have been variously recorded as symptomatic of BKD by others (Rucker et al. 1954; Earp et al. 1955; Snieszko and Griffin 1955; Bell 1961; Wood and Wallis 1955; Bendale and Klontz 1975; Bullock et al. 1975).

In a study which monitored changes in several hematological and blood biochemical parameters in juvenile chinook salmon (O. tshawytscha) during progressive BKD and exposure to NaPCP (Iwama 1977), the temporal development of kidney disease symptoms also was recorded for live fish surviving the experimental treatments. We report here a semi-quantitative description of the symptoms arising in progressive BKD, observations on mortality with progression of the disease, and further indication of synergism between chemical toxicity and a biological stressor in the form of a bacterial disease.

MATERIALS AND METHODS

Organisms

The fish were juvenile chinook salmon of length 11.5 ± 0.4 cm (mean ± SD) and weight 15.6 ± 2.0 g, obtained from Qualicum Hatchery on Vancouver Island, British Columbia. On arrival to the laboratory six groups of 240 fish each were transferred directly into 185 L tanks receiving well water (3.2 L/min). The fish were acclimatized for 27 days (12.0 - 12.2°C; O_2, 8.0-8.5 mg/L; pH 6.5-6.9; hardness, 54 mg/L $CaCO_3$) before the experimental treatments began. Food consisted of Oregon Moist Pellets presented daily throughout the experiment.

Kidney disease bacteria were obtained from the kidney of a moribund yearling (19 cm, 118 g) pink salmon (O. gorbuscha) from a stock with advanced

kidney disease. Approximately 0.7 g of kidney tissue was excised aseptically from the moribund salmon and homogenized in chilled, sterile 0.85% saline and 0.1% peptone (SP) solution. Cultures of the bacterial isolate were grown at 15 C on Evelyn's KDM II (Evelyn 1977). The SP bacterial suspensions were prepared from cells harvested after 14 days incubation and when in their log phase of growth (T. Evelyn, pers. comm.).

Toxicant treatment

Fish receiving the toxicant treatment were subjected to continuous-flow exposure to sodium pentachlorophenate (NaPCP) at one of two levels. The toxicant levels were 0.05 and 0.50 of the incipient 96h LC50 (0.078 mg/L) determined for juvenile chinook of the same stock and under conditions of high loading density (Iwama and Greer 1979). The toxicant was dispensed from modified Mariot bottles (Leduc 1966) at the flow rate of 0.04 L/min and was combined with the diluent water on entering the tanks. The combined flow of 3.2 L/min through each tank gave a 99.9% replacement time of approximately 4.5 h (Sprague 1969).

Disease treatment

Fish receiving the disease treatment were infected by intraperitoneal injection with approximately 41 x 10^6 viable kidney disease bacteria carried in a 0.1 mL suspension (0.10 optical density) of chilled SP solution. This bacterial dose was selected to obtain approximately complete mortality of test fish after an incubation time of about 40 days under experimental conditions that were similar to those in the incubation experiment with juvenile coho (Iwama 1980). The fish were injected in lots of 10-15 each following anesthetization in neutralized MS-222 (100 mg/L). The bacterial injections were carried out for all disease treated fish on the same day because it was uncertain the viability of the bacteria in suspension would remain constant for several days. Also on the same day and using identical procedures, all fish not receiving the disease treatment were sham treated with 0.1 mL injections of chilled SP solution. Fish were returned to their tanks following the injection procedures and given a 4-day period to recover from possible stress arising from the anesthesia and handling.

Treatment combinations

Three of the six groups of fish were injected with kidney disease bacteria. In two of these groups the disease treatment (D) was combined with exposure to the toxicant (T) at 0.05 and 0.50 of the 96 h LC50. In the sham treated fish, one control group (C) was maintained in diluent water and the other two groups were exposed to the 0.05 and 0.50 levels of the toxicant alone.

For brevity, the six treatment groups will be designated using the notations C, D; $0.05T_5$, $0.05T_5$+D; and $0.50T_6$, $0.50T_6$+D, where the semi-colon sets apart those group pairs sampled on the same day of a staggered 4-day sampling schedule. The order of the notations represents the treatment pairs sampled on the first-to-third days within a 4-day interval. The staggered schedule was necessary to enable in one day the completion of measurements on 10 parameters sampled for each treatment pair in the parent experiment (Iwama 1977). The schedule also provided one additional day for renewal of toxicant solutions and other preparations of apparatus and reagents.

The subscripts in the notations for toxicant treated fish denote the number of days the exposure to NaPCP lagged the disease and sham treatments, namely, five days and six days for the 0.05 and 0.50 levels of toxicant, respectively. Note that the toxicant treated fish had the same NaPCP exposure time on any given sampling day. The commencement of sampling of fish from the various treatments proceeded in the order of treatment notations given above on the 8th, 9th and 10th days, respectively, following the injection procedures.

Observations

Fish dying during the experiment were removed daily from the test tanks and the total mortality for each treatment group recorded for each 4-day sampling interval. Observations for external and internal symptoms of BKD were made on fish surviving the treatments. Twelve of the 24 fish comprising each sample in the parent experiment were used for the pathological examinations.

Before making the pathological examinations, kidney tissue for gram staining of smears was obtained aseptically with a sterilized innoculating loop inserted through an incision below and parallel to the lateral line. The incised area was prepared by removing the scales on one side and bathing the skin with 95% ethanol. The stained smears were examined for the presence of gram-positive diplobacilli which comprises presumptive diagnosis of the disease.

The presence and severity of external and internal physical characters symptomatic of the disease were noted for each fish and an integer index value assigned from Table I. Assignment of an index value from the table was based on the extent of the body features and tissues affected (across) and by judgement of the severity of symptoms present (down) compared to those characteristic of terminal stages of the disease. Table I was devised from symptoms of BKD described by others (e.g. Rucker et al. 1954, Earp et al. 1955, Sneiszko and Griffin 1955, Bell 1961, Bullock et al. 1975), from unpublished observations of pathological condition noted at time of death in juvenile coho similarly infected with kidney disease bacteria (Iwama 1980) and also from observations made in the present study.

Post-mortem examination was made of all fish dying during the experiment. However, a symptomatic index was not assigned nor were kidney smears prepared for dead fish since post-mortem changes possibly occurring within 24 h could resemble some of the symptoms of BKD (e.g. accumulation of abdominal fluid, pale color of organs, tissue lysis).

Data analysis

The mean symptomatic index was calculated for each sampling interval using 12 surviving fish from each treatment.

The mortalities occurring over each 4-day sampling interval were expressed as cumulative percent of the original sample size (N = 240). Since a total of 24 live fish were removed from each treatment at the sampling intervals, plots depicting the cumulative percent mortality describe truncated mortality curves. This distinction is made in Results and Discussion by using the term mortality index.

RESULTS AND DISCUSSION

Behaviour appears to be a generally sensitive indication of stress or other dysfunction and note was made of the treatment groups first showing changes in feeding behaviour and also the behaviour of the aggregate form of the fish which, in the tanks, resembled that of a school. During acclimatization, the fish appeared to adapt well to the high loading density (20 g/L) and readily took food within one week of transfer to the tanks. A reduction in feeding behaviour was observed only in groups D and $0.05T_5$+D and first occurred 12-13 days post-injection. Deterioration in organization of the aggregate form did not occur in groups C and $0.05T_5$ but was first noted in group $0.05T_5$+D 17 days post-injection and in groups D and $0.50T_6$ 20 and 34 days post-injection, respectively. In association with the changing aggregate behaviour, a few mortalities were beginning to occur in those treatment groups and some of the surviving individuals lacked alertness, appeared disoriented, or showed little resistance to capture by net.

There was little opportunity to observe behaviour of the fish in treatment group $0.50T_6$+D because the 216 fish remaining after the first sampling on the 10th day post-injection died on the 13th - 14th days post-injection. It was noted on the 13th day that many fish were at the water surface but feeding behaviour was still normal. The surfacing behaviour appeared not to be related to low oxygen concentration since determinations made in the morning and afternoon preceding the sudden deaths were in the usual range 8.0-8.5 mg/L. Although these mortalities were an unexpected event, it had precedent in an earlier experiment of identical design but using juvenile coho (unpub. obs.). In that experiment, which was shortly terminated because it was thought an error had been made in the toxicant concentration or in some other procedure, the coho died over several days between the 10th and 14th days post-injection. The similar outcome with juvenile chinook substantiates the marked synergism between the combined stressors BKD and NaPCP at 0.50 the 96 hr LC50.

There were no mortalities in the control group. The time-course of mortalities for the other treatments is depicted by the mortality index curves in Fig. 1 (bold lines). Mortalities in the groups receiving the toxicant only ($0.05T_5$ and $0.50T_6$) were modest and in the low toxicant exposure consisted of three fish. Exposure to NaPCP at 0.05 the 96 h LC50 had little additive effect on mortalities arising from the disease alone ($0.05T_5$+D and D; Fig. 1).

Progression of the symptoms of BKD in fish receiving the disease treatments is also presented in Fig. 1 (lighter lines). No symptoms of BKD were observed in fish from the other treatments. Note that a symptomatic index could be determined for only one sample of fish surviving the $0.50T_6$+D treatment. Gram-positive diplobacilli in kidneys of surviving infected fish first appeared on the 12th day post-injection. Although gram staining of kidney smears and assignment of a symptomatic index was not done for disease-treated fish that died during the sampling intervals, pathological examination of those fish indicated that BKD was progressing at a rate similar to that in the fish surviving the disease treatments at corresponding sampling intervals. There were large variations (not shown) associated with

the mean values of symptomatic index indicating that progression of the disease was not different between disease and disease plus toxicant treatments. However, because the symptoms were assessed at unequal times following bacterial injection, the days post-injection become implicit, additional treatment levels of the disease factor which possibly contributed to the apparent higher values of symptomatic index for the $0.05T_5$+D group and for the one determination made for the $0.50T_6$+D group, compared to group D (Fig. 1). It seems unlikely therefore, that exposure to NaPCP influenced the course of the disease in injected fish.

At approximately 21-24 days post-injection an inflection in the curves for symptomatic index can be discerned which was concurrent with a marked increase in mortality of fish in the same treatment groups (Fig. 1). Symptoms at this stage of the disease corresponded to an index of 3-3.5 (Fig. 1) and would be manifest in external features consisting of a bloated abdomen and, in some individuals, the appearance of yellowish fluid exuding from the vent (Table I). The fish dying suddenly at about 14 days post-injection in the $0.50T_6$+D group were only moderately symptomatic however, having a mean index of about 2 and external features of the disease which might not be easily distinguished from fish that simply were well fed. There were no mortalities at a similar number of days post-injection in fish receiving the disease treatment alone or in combination with NaPCP at 0.05 the 96 h LC50 (D, $0.05T_5$+D; bold lines, Fig. 1). Thus it seems there could exist a number of combinations of NaPCP toxicant level and state of progression of bacterial kidney disease which would constitute interactive thresholds leading to increased mortality.

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Table I. Index of symptoms in juvenile chinook salmon infected experimentally with kidney disease bacteria.

	External			Internal							
Symptomatic index	Abdominal form	Vent	Body surface	Fluid in abdominal cavity	Peritoneal surface	Kidney	Hind gut	Liver	Gonadal tissue	Spleen	Swimbladder
1				some							
2	slightly distended			some	some hemorr.[a]	slightly enlarged	slightly enlarged				
3	distended			moderate	hemorr.	enlarged	enlarged	hemorr.	hemorr.		
4	distended	some yellowish exudate		moderate	hemorr.	enlarged; small lesions	enlarged	hemorr.	hemorr.		
5	distended	some yellowish exudate	few unbroken welts	excessive	hemorr.	swollen; pale color; lumpy with lesions	enlarged	hemorr.	hemorr.	enlarged; pale color	smokey appearance
6[b]	distended	yellowish exudate; hemorr.	broken welts; petechiae	excessive excessive	hemorr. hemorr.	swollen; pale color; lumpy with lesions	enlarged	hemorr.	hemorr.	enlarged; pale color	smokey appearance

[a]hemorr. = hemorrhaging
[b]at this stage, internal hemorrhaging widespread; autolysis evident in some tissues.

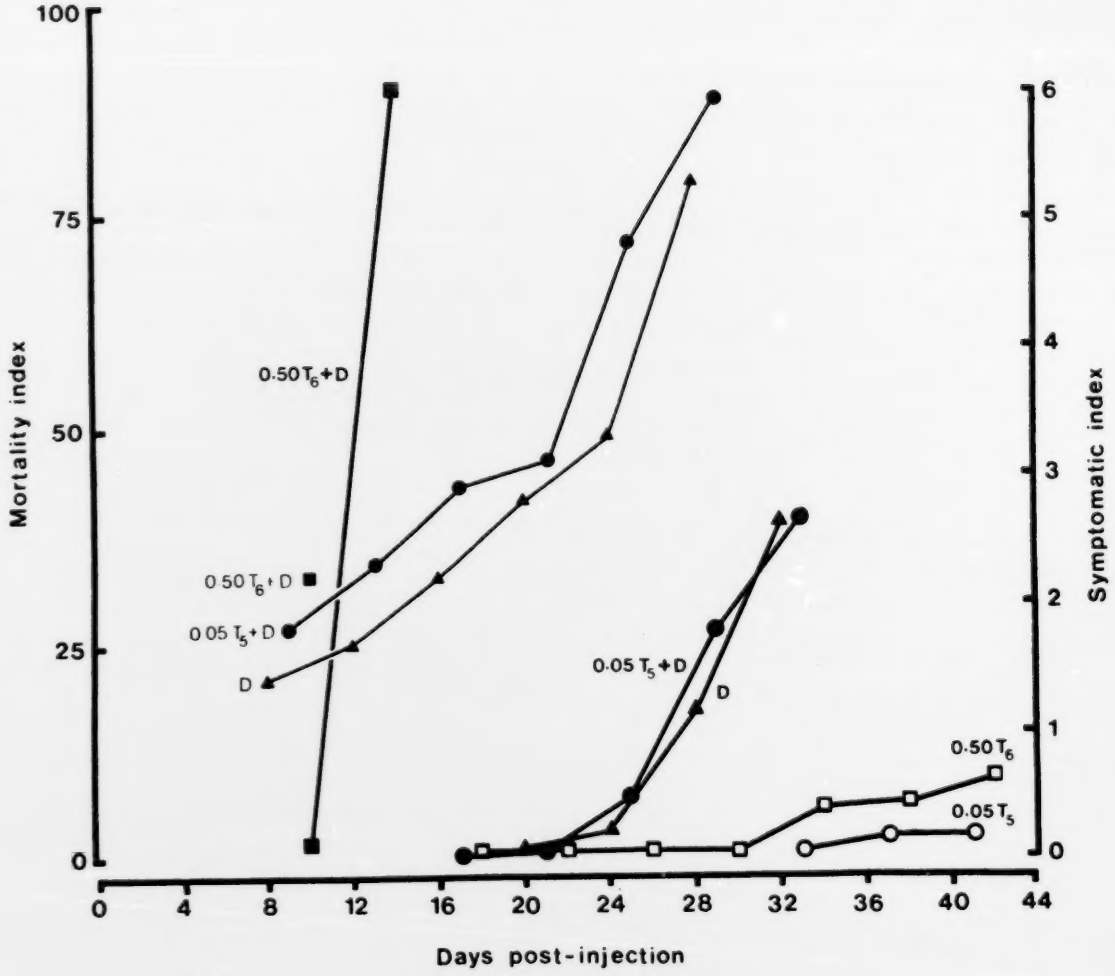

Fig. 1. Mortality (bold lines) and progression of kidney disease symptoms (lighter lines) in juvenile chinook salmon treated with sodium pentachlorophenate (T) at 0.05 or 0.50 the 96 h LC50 and intraperitoneal injections of kidney disease bacteria (D), singly and in combination. Subscripts in the treatment notations (T_5,T_6) denote the number of days the toxicant exposure lagged the bacterial or sham injections.